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Manual

rapidSTRIPE Borrelia Assay



Order No.:

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845-IS-1002025 25 reactions

845-IS-1002050 50 reactions

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1 Introduction

The arising warm climate in middle Europe will lead to a dramatic increasing of ticks. Referring to this fact, also the risk of a tick bite will be increased. Ticks are carrier of infectious pathogens and induce different diseases (zoonoses).

Borrelia burgdorferi, the pathogen of the Lyme disease belongs to the best known bacteria, which are widespread by ticks. The infestation rate of ticks in this country averages approx. 11 – 34 %. In Germany are about 60.000 incidences per year. The probability to contract after a tick bite is approximately 1 – 5 %. Thereby the risk of infection will be reduced by an early removal of the tick, because normally the transfer of the pathogens will occur after 24 h of a suction process.

The Lyme – disease is a progress, which typically consists of 3 phases.

After an incubation time of approx. 3 – 32 days phase I, which lasts about 2 – 6 weeks, starts with a Erythema chronicum migrans, accompanied with general symptoms, fever and Lymphadenitis. In 90 % of all cases this infection heals up without an antibiotic therapy.

In approx. 10 % of the patients the phase II (phase of dissemination) is followed after 2 till 10 weeks with an involvement of organs, like Mono- or Oligoarthritis, Myo- and Perikarditis, Meningitis, Meningoradikulitis (Bannwarth – Syndrome), Facialisparesis, Chorioretinitis, Uveitis or Optikusneuritis. In 99 % of all cases a spontaneous remission occurs after weeks and month respectively.

2 Test description and principle

The rapidSTRIPE Borrelia Assay is a molecular diagnostic test system to determine Borrelia DNA in ticks.

The rapidSTRIPE Borrelia Assay detects the nucleic acids of the pathogens directly inside the sample material (ticks). The test contains of 3 modules, which functions are optimized to each other.

2.1 Module nucleic acid purification

Module nucleic acid purification is used for the isolation of nucleic acids from the sample material (tick tissue). Thereby it is possible to extract only DNA (blackPREP Tick DNA Kit) or to process a simultaneous isolation of DNA and RNA from the tick tissue (blackPREP Tick DNA/RNA Kit).

The blackPREP Tick DNA Kit is used to isolate DNA from ticks and is applicable for the detection of bacteria and protozoa inside the tick tissue. The blackPREP Tick DNA/RNA Kit is used for the simultaneous isolation of DNA and RNA directly from ticks. This is of especially interest, if next to the analysis of bacterial pathogens, also e.g. RNA viruses have to be determined (e.g. detection of TBE).

In both extraction kits, first the tick has to be mechanically homogenized using e.g. SpeedMill (Analytik Jena AG) or other commercially available homogenizers on the basis of beads.

After the mechanical homogenization, the lysis or denaturation of the sample is followed. Consecutively the released nucleic acids are specifically bound onto a spin filter surface, washed and finally eluted. Now the nucleic acids are ready for any further downstream application.

2.2 Module PCR amplification / hybridization

Module PCR amplification / hybridization is used for the detection of the *Borrelia* – specific DNA. The isolated DNA is used for a *Borrelia* species amplification reaction. The amplification is in the following combined with a hybridization reaction using a *Borrelia* specific probe within the same well of the PCR plastic. This reaction format allows thus a specific determination of the *Borrelia* DNA and avoids false negative results because of a mispriming.

The amplification protocols are optimally adapted to the unique rapidPCR technology using Low Profile Rapid (LPR) blocks or Standard Profile Rapid (SPR) blocks (Analytik Jena AG), as well as optimized to the usage of standard PCR thermal cyclers, e.g. FlexCycler (Analytik Jena AG). Thereby the advantage of the rapidPCR technology is given by a duration of the test performance in less than 1 hour. Each kit contains special PCR microplates or strips for the rapidPCR and 0.2 ml 8 well strips for standard PCR.

2.3 Module detection

Module detection is used to visualize and to analyse the amplification – hybridization results by a user-friendly Lateral Flow Strip. To visualize the reaction the amplification mix will be transferred onto a Lateral Flow Strip. A positive PCR/hybridization result will be confirmed by a visible test line.



Attention

Results from ticks may not be adducted as exclusive basis for further therapies!

3 Performance assessment, spectrum of application and specificity

This test was used for the analysis of about 400 ticks to determine the occurrence of *Borrelia* infections. Positive test results were sequenced consecutively to verify the results and to classify *Borrelia*. The following tick species were analysed:

- Dermacentor reticulatus
- Ixodes hexagonus
- Haemaphysalis concinna
- Ixodes ricinus

The following table gives an overview of the results, which were determined in this study.

Tick species	Number		Borrellia species (determined by sequencing)
	Complete	Borrellia positive	
Dermacentor reticulatus	36	2	B. afcelii
Ixodes hexagonus	1	-	
Haemaphysalis concinna	5	-	
Ixodes ricinus	358	56	B. afcelii, B. garinii
Complete	400	59	

4 Kit components, storage and stability



Important!

The master mix has to be prepared freshly before each application. Storage of a ready-to-use master mix could lead to false positive results due to the formation of primer – probe – dimers.












Note!


Only for Module PCR amplification / hybridization and Module detection and the usage of 36, 96 well microplates LP, 8 well strips LP or 8 well strips 0.2 ml.

Kit components and volumes or amounts are listed in the component table below. All components are ready to use and stable until expiry date mentioned on the kit packaging, if stored as specified in the following.

Module PCR amplification / hybridization

Component		Content per reactions			Storage
		10	25	50	
8 well strip LP with sealing foil		3	5	10	Packed within Module detection
8 well strip 0.2 ml with lid		2	4	7	
<i>Borrelia</i> positive control		7.5 µl	15 µl	25 µl	- 20 °C
Primer 1 BO		25 µl	60 µl	100 µl	- 20 °C
Primer 2 BO		25 µl	60 µl	100 µl	- 20 °C
Probe BO		25 µl	60 µl	100 µl	- 20 °C
dNTP mix		10 µl	20 µl	40 µl	- 20 °C
10x SpeedAmp PCR Buffer		50 µl	100 µl	200 µl	- 20 °C
10x PCR Buffer		50 µl	100 µl	200 µl	- 20 °C
PCR-grade H ₂ O		250 µl	500 µl	1000 µl	- 20 °C
innuTaq HOT DNA Polymerase		10 µl	15 µl	20 µl	- 20 °C work on ice

Module detection

Components		Content per reactions			Storage
		10	25	50	
Lateral Flow Strips		10	25	2x 25	4 °C close airproof
Running buffer		2 ml	5 ml	10 ml	4 °C
Sample Tubes (2.0 ml)		10	25	50	Room temperature

5 Necessary laboratory equipment and additives

- SpeedMill (Analytik Jena AG) or other commercial available homogenizers on the basis of beads
- rapidPCR thermal cycler with a Low Profile Rapid (LPR) block / Standard Profile Rapid (SPR) block (Analytik Jena AG) or a standard PCR thermal cycler with heated lid and 0.2 ml wells (e.g. FlexCycler, Analytik Jena AG)
- Microcentrifuge
- Vortexer
- Variable pipettes for 10 µl, 100 µl and 1.000 µl (use separate pipettes for extraction, amplification and detection)
- Sterile pipette tips with protection against contamination (filter tips)

6 Remarks and safety precautions

All reagents in this kit only have to be used for the intention mentioned inside the user manual. The application may only be exercised by authorized personal.

During the operation, the described protocol has to be followed strictly. Furthermore the regularities to operate quality controls within medical laboratories have to be considered.

The reagents should be stored inside the original vessels at the mentioned temperatures. Single components of different charges and consumables may not be exchanged. The mentioned expiry dates have to be considered.

The material to be determined has to be categorized as potential infectious. The accordant precautions have to be noticed.

For the exposure to the kit reagents and the sample material, the accordant regulations to prevent accidents for the medical service have to be observed. Particularly the following precautions have to be considered:

- Don't eat, drink or smoke!
- Always wear protective clothing and gloves!

The reagent vessels could be disposed with the normal laboratory waste.

7 Performance of the test



Important notes!

- Do not exchange the components of different kits or kit charges
 - Open and close the vessels of single components always separately
 - Change contaminated gloves immediately
 - Spatial separation of the amplification and detection area
 - Perform the procedure in the order of the following steps:
 1. Sample preparation / nucleic acid extraction
 2. Amplification and hybridization
 3. Detection
 - Do not open PCR plastics, which contain amplified samples in the area of sample preparation (NA isolation) or preparation of amplification
 - Amplified samples and controls are potential sources of contamination
 - Use separate pipettes with sterile filter tips for the preparation of the PCR reaction master mixes
 - Open the reaction vessels carefully to avoid the generation of aerosols
-

7.1 Nucleic acid isolation

The isolation of the nucleic acids has to be done using the blackPREP Tick DNA Kit or the blackPREP Tick DNA/RNA Kit. The protocols inside the accordant user manual have to be followed exactly.

Note: The operation of the test was optimized by using nucleic acids, which were isolated by the above mentioned extraction kits. Alternatively, also nucleic acids, which were isolated by other methods could be used.

7.2 PCR amplification / hybridization



Note

- Only for application of Module PCR amplification / hybridization
 - For usage of 36, 96 well microplates LP, 8 well strips LP or 8 well strips 0.2 ml
-

The performance of the amplification and the hybridization of the PCR product could be done either using a rapidPCR thermal cycler, as well as using a standard PCR thermal cycler (including a heated lid). The thermal cycler also needs a sample protection system (SPS) that cools samples to the set temperature (105°C – 120°C) while the lid is heating in order to prevent primer/probe dimer formation, non-specific annealing and early elongation.

7.2.1 Initial steps

1. Divide the DNA eluates and controls to the accordant PCR plastic

	8 Well Strip LP	8 Well Strip 0,2 ml
Sample (extracted DNA)	1.5 µl	2.5 µl
Positive (positive control)	1.5 µl	2.5 µl
Negative (PCR-grade H ₂ O)	1.5 µl	2.5 µl

2. The prepared plastic has to be stored on the cooling block until the amplification is started

7.2.2 Preparation of the PCR reaction mix



Important!

The master mix has to be prepared freshly before each application. Storage of a ready-to-use master mix could lead to false positive results due to the formation of primer – probe – dimers.

1. Thaw all reagents of Module PCR amplification / hybridization, vortex, spin down and store the components on ice during the preparation
2. The preparation of the master mix for one sample is described in the following table. The preparation of the master mix has to be done for the number of used samples (including positive and negative controls)

Mastermix	rapidPCR		standard PCR
Plastic	8 well strip LP (20 µl)	8 well strip (0.2 ml)	8 well strip (0.2 ml)
10x SpeedAmp PCR Buffer	1.5 µl	2.5 µl	-
10x PCR Buffer	-	-	2.5 µl
Primer 1 BO	1.0 µl	1.5 µl	1.5 µl
Primer 2 BO	1.0 µl	1.5 µl	1.5 µl
Sonde BO	1.0 µl	1.5 µl	1.5 µl
dNTP Mix	0.3 µl	0.5 µl	0.5 µl
innuTaq Hot DNA Polymerase	0.15 µl	0.25 µl	0.25 µl
PCR-grade H ₂ O	8.55 µl	14.75 µl	14.75 µl
Final PCR volume	15 µl/reaction	25 µl/ reaction	25 µl/ reaction

3. The master mix has to be added to the wells, which still contain the pre-pipetted DNA samples (positive and negative controls respectively) as described in the following

	rapidPCR		standard PCR
Plastic	8 well strip LP (20 µl)	8 well strip (0.2 ml)	8 well strip (0.2 ml)
Master mix	13.5 µl	22.5 µl	22.5 µl
Finale PCR volume	15 µl/reaction	25 µl/ reaction	25 µl/ reaction

4. Seal the PCR plastic with the accordant foil (PP), put it into the thermal cycler and close the lid
5. Start the PCR time and temperature protocol

7.2.3 Amplification and hybridization

The PCR protocol contains two steps:

- Step 1: Amplification and labelling of the *Borrelia* specific DNA fragment.
- Step 2: Hybridization of the amplified DNA sequence using the *Borrelia* specific probe.



Attention!

The following PCR protocols are adapted to the accordant PCR thermal cycler.

rapidPCR thermal cycler with LPR or SPR block:

Amplification and hybridization

Step	Cycle	Profile	Temperature	Holding time	Ramp rate
1	1	Initial denaturation	95 °C	120 sec	max
2	42	Denaturation	95 °C	4 sec	max
		Annealing	57 °C	4 sec	max
		Elongation	72 °C	20 sec	max
3	1	Denaturation	95 °C	300 sec	max
		Hybridization	45 °C	650 sec	max

Standby: 18 °C

Time: approx. 47 min

Standard PCR thermal cycler:**Amplification and hybridization**

Step	Cycle	Profile	Temperature	Holding time	Ramp rate
1	1	Initial denaturation	95 °C	120 sec	max
2	42	Denaturation	95 °C	30 sec	max
		Annealing	57 °C	30 sec	max
		Elongation	72 °C	60 sec	max
3	1	Denaturation	95 °C	300 sec	max
		Hybridization	45 °C	650 sec	max

Standby: 18 °C**Time:** depending on thermal cycler

7.3 Detection

7.3.1 Introduction

The determination of the combined amplification / hybridization reaction is done by visualization on a Lateral Flow Strip (fig. 1). The Lateral Flow Strip consists of the following areas:

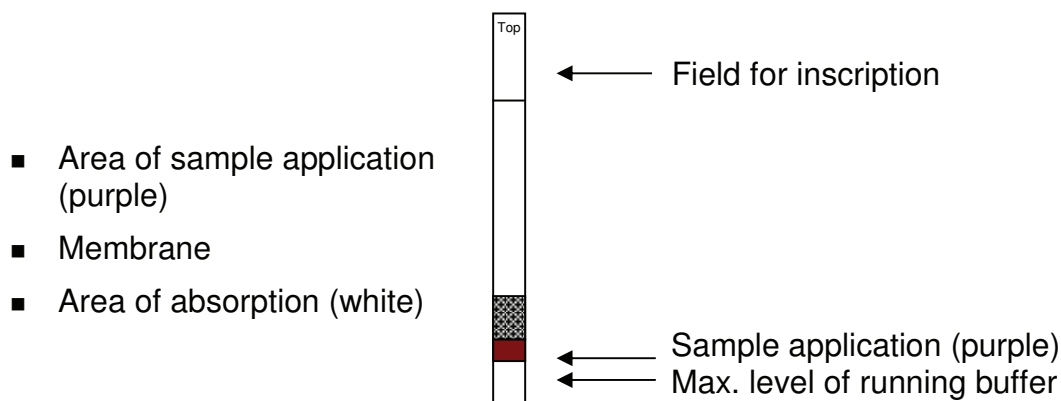


Fig. 1: Design of the Lateral Flow Strip

The whole Lateral Flow Strip, besides the lower part of the sample application area, is covered with a foil and can be touched on this foil. The foil above the absorption area can be used for any inscriptions. After the test is finished, the Lateral Flow Strips can be archived in a progress report.

7.3.2 Performance

1. Take the needed number of Lateral Flow Strips out of its package, inscribe it and place it ready

Note: Only areas, which are covered with a foil can be touched and inscribed. Store the residual Lateral Flow Strips closed under adequate conditions.

2. Apply 10 µl of the PCR / hybridization reaction on the head of the sample application area (fig. 1, purple) at the border of the foil and incubate for at least 1 minute at room temperature.

Note: Thereby the occurrence of a smear is normal.

3. Add **150 µl Running Buffer** to each single 2.0 ml Sample Tube
4. Place the Lateral Flow Strips with the membrane into the prepared 2.0 ml Sample Tubes and incubate until the area of sample application is discolored (approx. 20 min).

8 Analysis

The test is valid, if for each determined sample (positive control, negative control and sample) a colored control line is visible (fig. 2).

For each current test performance, the accordant positive and negative controls have to be correct. In case of the PCR negative control the test line has to be invisible (fig. 2B). If the test line of these samples is visible, the analysis for all tested samples has to be repeated.

1. **Two red lines are visible:**
(Test and control line)

The sample is **positive** (fig. 2A).



Attention

Also a light colored test line has to be valued as positive. Compare with the negative control. If necessary repeat the whole test to confirm the result. The intensity of the control line has no influence on the result validation, because the control line is always more intensive in comparison to the test line.

Positive results can be visible before the incubation is finished.

2. **Only one red line (level of the control line) is visible:**

The sample is **negative** (fig. 2B).

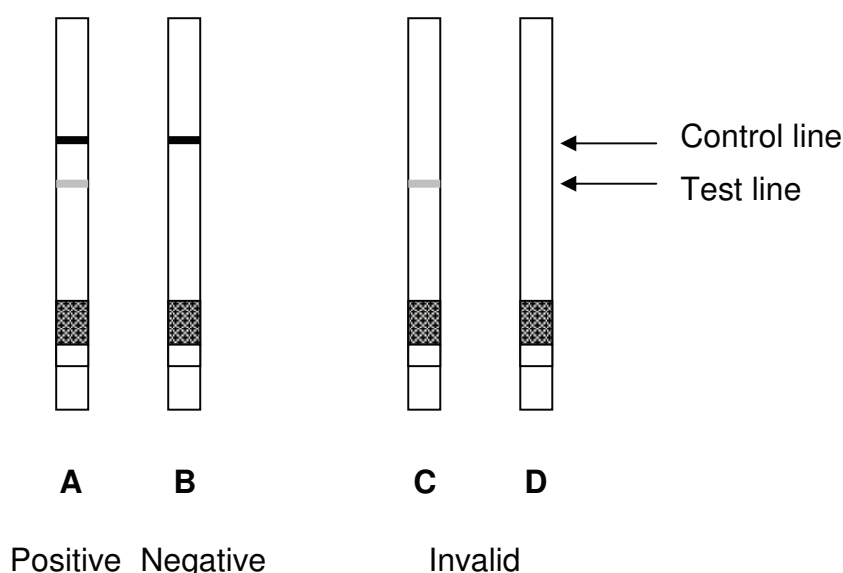


Fig. 2: Analysis of the reaction on the Lateral Flow Strip

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